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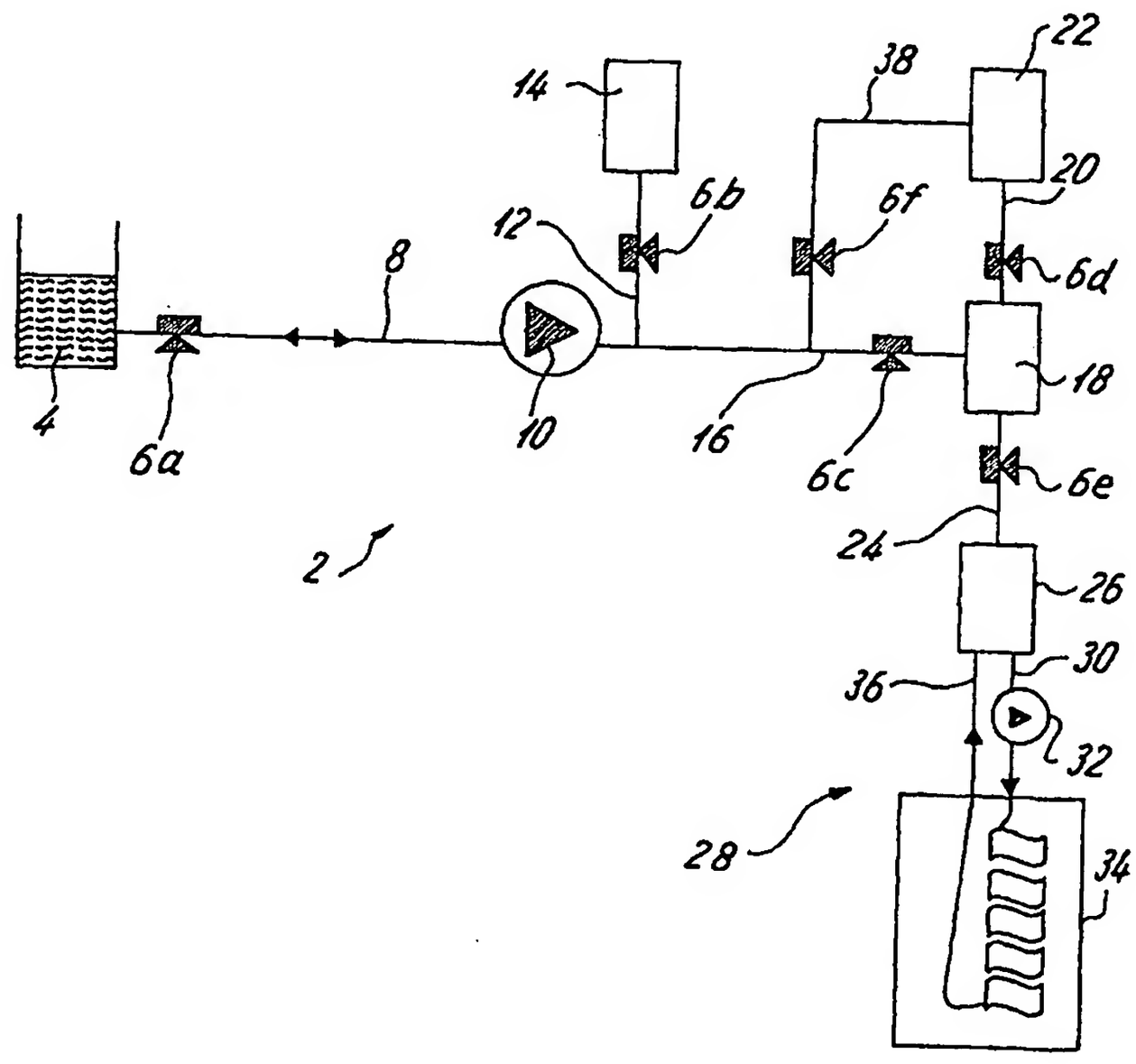
(54) Title: PHOTODYNAMIC INACTIVATION OF VIRUSES IN BIOLOGICAL FLUIDS

(57) Abstract

A device and a process for the inactivation of viruses in biological fluids, especially for the inactivation of viruses — especially HIV — in blood and blood products in which phenothiazine dyes, especially toluidine blue or methylene blue, are added to the biological fluid; a single–part or multipart pumping and transport device is used to collect the biological fluid from a container or circulation, the biological fluid passing through a system of conduits and returning to the container or to the circulation. A separating device serves to separate the biological fluid into a liquid and a corpuscular phase. A light source emits electromagnetic radiation of the visible spectrum onto a light–permeable segment of the conduit system.



However, this increase in the cross-sectional area must be balanced. If the increase is too great, the radiation may not reach all of the fluid in the conduit. Therefore, the widening of the conduit must be tempered. Alternatively, a second transparent area may be introduced to allow for greater phototherapy. In such a case, the same light source may be used, or an additional light source may be added.

For simpler manipulation in another variant a vein adapter or sensor can be connected preferably to the reservoir interface. A temperature regulating device wired into the conduit system for regulating the temperature of the biological fluid makes the necessary cooling of the blood possible.

The device preferably includes a metering device connected into the conduit system for releasing the dyes into the biological fluid.

The invention also creates a new application for known drugs, that being the application of phenothiazine dyes, especially toluidine blue or methylene blue, for the production of a drug for the inactivation of viruses in a therapy process of the type explained below.

The invention also creates a process for inactivation of viruses in biological fluids, especially for the inactivation of viruses -- especially HIV -- in blood and blood products, which involves adding photosensitizers to the biological fluid, preferably phenothiazine dyes, especially preferably toluidine blue or methylene blue, with the following steps: the biological fluid is separated by a separating device into different liquid phases and/or liquid and solid phases, especially into blood plasma and corpuscular elements, one of the liquid phases (i.e. plasma) is bombarded with electromagnetic radiation, especially of the visible spectrum, until part of the viruses present in the blood has been essentially destroyed or deactivated, and the liquid and/or solid phases are brought together again after irradiation and/or mixed with each other again. The irradiation is performed on the blood fraction which is free, or substantially free, of red corpuscles to prevent destruction of those cells.

to the conduit 6 with which the blood can be pumped in the conduit 8 in both directions. Behind the pump 8 a conduit branch 12 with a valve 6 makes it possible to add an anticoagulant from a feeder device 14 designed for this task. The feeder device 14 or another (not shown here) feeder device may, if necessary, also be utilized for the direct addition of the thiazine dyes to the blood of the patient.

From the pump the pumped-in blood is fed via a third valve 6c and a conduit branch 16 into a centrifuge or a filtration device 18 (e.g., a PRISMA™ CFR microfiltration unit trademark of HOSPAL Medizintechnik GmbH, Brettergartenstr. 16 90477 Nuremberg, Germany). The centrifuge or the filtration device 18 makes it possible to separate the fed-in blood into two liquid phases and/or liquid and solid phases. One of these phases (with a high content of red blood corpuscles) is sent via the branch line 20 with a valve 6d to a return pouch 22 or directly back to the container 4 or a patient's circulation. The other phase is sent via a branch line 24 with a valve 6d to a plasma container 26. From the plasma container 26 the blood plasma is then sent through an irradiation cycle 28 with inflow line 30, pump 32, irradiation unit 34 with (not shown) cooling and return line 36.

Finally the blood is returned from the plasma container 26 via the centrifuge or filtration device 18 and the return pouch 22 and a return branch line 38 with valve 6f as well as the pump 10 and conduit 8 with the valves in the appropriate position to container 4 or to the blood circulation of a patient.

The example in Figure 2 differs from this example of embodiment essentially in the fact that the blood is passed from the pump 10 directly to a centrifuge/irradiation unit combination 40 where the lamp segment 42 emits light onto the centrifuge part 44 with walls with light-permeable segments.

An exemplary treatment with the above-described device follows:

For a quantity of 500 ml plasma one should use between 0.005 and 0.025 ml, preferably 0.010 to 0.020 ml, most preferably approximately 0.015 ml of 1% sensitizer. The photosensitizer is most conveniently in an aqueous solution of

bound. Ultimately this results in the denaturing of the components of the virus shell or breaks in the strands of the viral HIV RNA. Highly reactive oxygen radicals are also involved in this process. It has also been demonstrated that the dyes interfere with transcription and translation (of DNA and RNA) in darkness as well as in a weak or a strong light. Preferably the plasma is irradiated for 1 hour by the light source (50,000 lux). However the time necessary for destruction or inactivation of viruses will depend on many factors including the particular virus, the dye concentration, the particular dye used, the temperature, the light intensity, and the wavelength used, among others. This process is temperature dependent, i.e. the operating temperature must be between 20 and 30°C so that any proteins still present are not destroyed. After the irradiation time the plasma is reinfused back into the patient. The treatment (collection and irradiation process) could be performed initially several times daily. The therapy cycle is repeated, depending on the virus load of the patient, from initially, e.g., five times weekly to once per month.

The invention is now illustrated by non-limiting, representative examples.

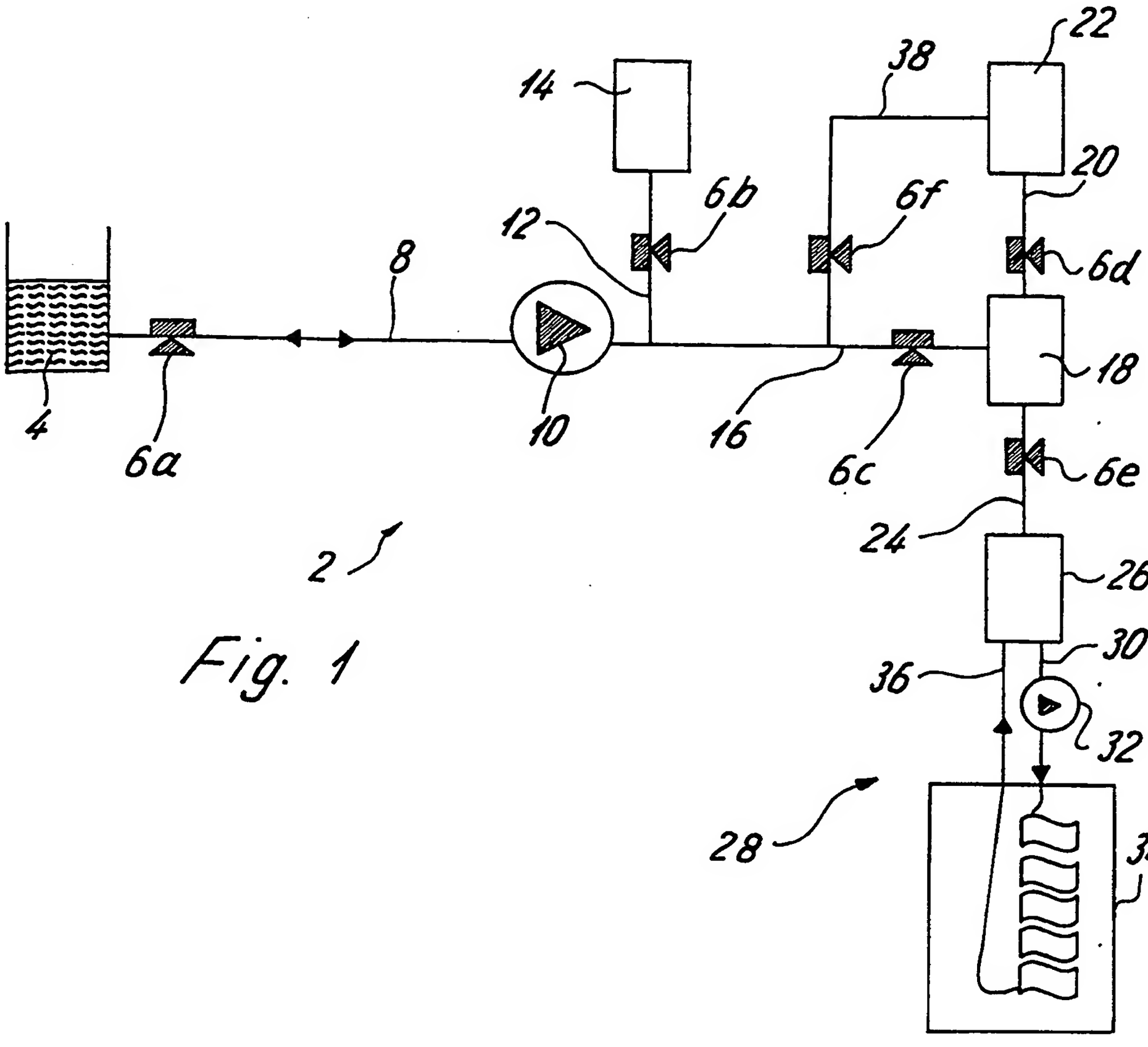
Example 1

This experiment shows the virus inactivation properties of methylene blue and light in plasmas from four different patients.

TABLE 1

Irradiation time/minutes	HIV copies/ml without methylene blue	HIV copies/ml with Methylene blue
0	814	Below detection limits
60	743	Below detection limits
60	10751	Below detection limits
60	9275	Below detection limits
60	3944	Below detection limits

With the aid of the AMPLICOR HIV-1 Monitor Test (version 1.5) from Roche the HIV RNA in the treated plasma could be detected quantitatively. HIV was found to be especially susceptible to the photo-inactivation step.


14
38
22
20
6b
6f
12
6d
8
18
10
16
6c
6e
4
6a
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26
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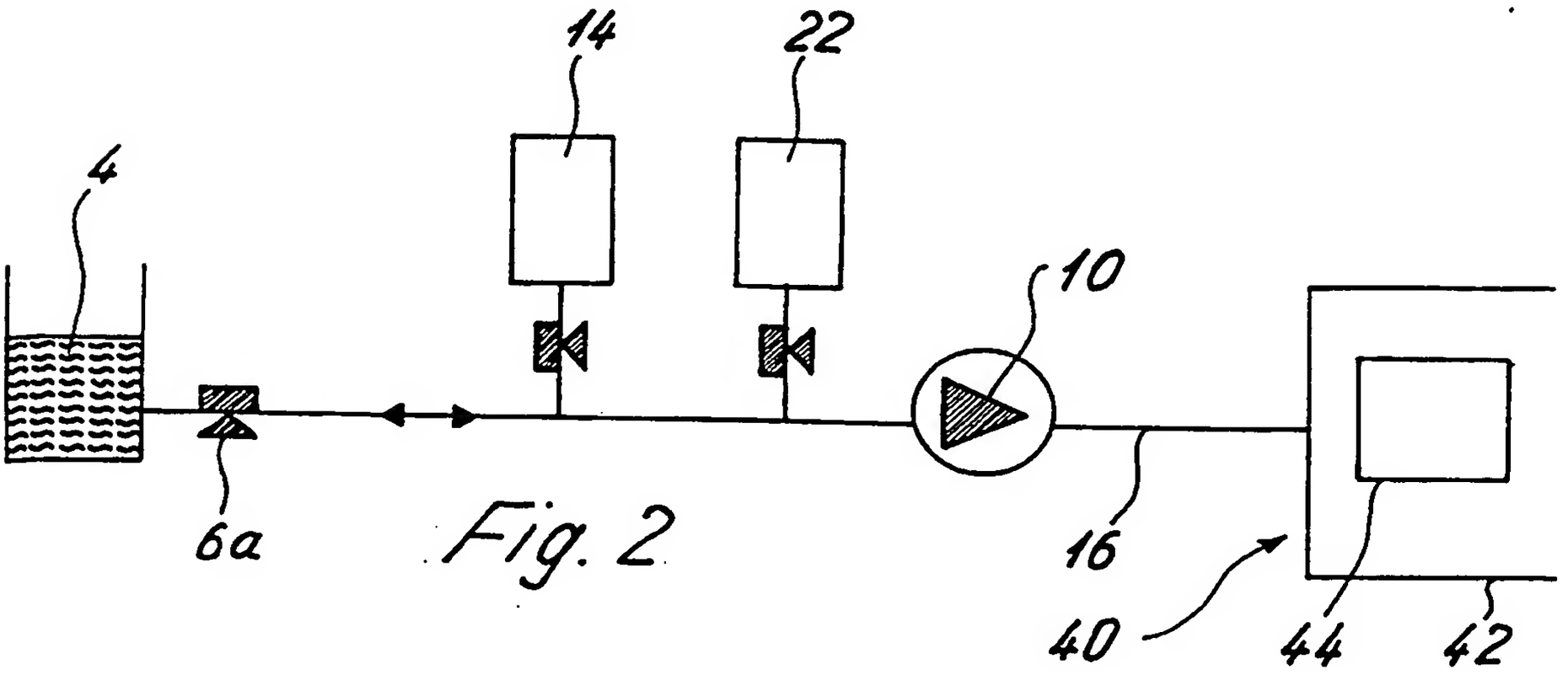
36
32
28
34

Fig. 1


14
22
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10
6a
16
40
44
42

Fig. 2